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Ovine bladder smooth muscle cell morphology and adhesion on coverslips coated with or without proteins. Thirty minutes prior to cell seeding, serum free DMBM and DMEM supplemented with 10% FBS and 1% P/S were placed onto the protein coated substrates and reference substrates, respectively.

Bladder smooth muscle cells were enzymatically lifted from polystyrene tissue culture dishes using 1 ml of PET and resuspended in serum free DMEM. Cells were seeded (3,500 cells/cm²) onto each substrate and incubated under standard cell culture conditions for 4 h.

At the end of each time period, non-adherent cells were removed by gentle rinsing in PBS. Cells adherent to the substrates were fixed with 10% formalin, stained with Coomassie Brilliant Blue for 10 minutes, and rinsed with PBS. Cell morphology was examined using light microscopy (Nikon Microphot-FXA; Nikon, Japan) and digital imaging (Flashbus FBG 4.2; Integral Technologies, Inc.). In addition, the number of cells in each of five random fields per substrate were counted using light microscopy (Olympus CR2; Olympus, Germany), averaged, and reported as cell density (cells/cm²). All experiments were run in triplicate and repeated a minimum of three separate times.

Bladder smooth muscle cells adherent on glass coverslips coated with collagen type IV in the presence of serum free DMEM exhibited superior spread morphology compared to those adherent on glass coverslips coated with vitronectin and collagen type III, which exhibited superior morphology to those adherent on the albumin-coated control coverslips.

In addition, collagen type IV enhanced bladder smooth muscle cell adhesion compared to the albumin control coverslips. Cell adhesion was significantly (p < 0.05) increased on coverslips coated with gelatin, fibronectin, vitronectin, laminin, and collagen type I, and was significantly (p < 0.01) increased on glass coverslips coated with collagen type IV, after 4 h of incubation. The adherent cell density on glass coverslips coated with collagen type IV was 762%, 324%, 148%, 132%, 96%, 86%, and 58% higher than on glass coverslips coated with laminin, collagen type I, albumin, fibronectin, collagen type III, vitronectin, and gelatin, respectively.

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L7	2	"6689374".pn.	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	ON	2007/05/23 08:02
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L12	22	19 and 111	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR .	ON	2007/05/23 09:05

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